

University of Groningen

Brain dead donor graft deterioration and attenuation with N-octanoyl dopamine preconditioning

Hottenrott, Christina Maximilia Valentina

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:
2016

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Hottenrott, C. M. V. (2016). *Brain dead donor graft deterioration and attenuation with N-octanoyl dopamine preconditioning: Emphasis on lung and kidney grafts*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen.

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

CHAPTER 7

Quality of donor lungs: A comparative study between explosive and gradual brain death induction models in rats

Maximilia Hottenrott
Rolando Rebolledo
Dane Hoeksma
Jeske Bubberman
Johannes Burgerhof
Annette Breedijk
Benito Yard
Michiel Erasmus
Henri Leuvenink

Manuscript in preparation

Abstract

Despite the fact that brain death induces pro-inflammatory changes, correlating with the reduction of graft quality and outcome after transplantation, brain dead donors are the major source for transplantation. This study is designed to test whether explosive or gradual increase in intra cranial pressure, to induce brain death, have a differential effect on the graft quality, and to identify deleterious mechanisms during brain death.

Fisher (F344) rats were randomly assigned into three donor groups: 1) no intervention and immediate sacrifice, 2) explosive - and 3) gradual brain death induction model, the latter were subdivided in sacrifice time points 30 minutes, 1 hour, 2hrs. and 4 hrs. after brain death induction. During the brain death period the animals were hemodynamically stabilized (MAP > 80 mmHg) and lung protective ventilated (VT = 6.5 ml/kg of body weight and a PEEP of 3 cmH₂O). Hemodynamic changes and pulmonary inspiratory pressure were monitored, the lungs (n = 8/group; excluding lost animals) were analyzed with a histological scoring system and for pro-inflammatory changes in gene expression with polymerase chain reaction.

Immediately after explosive traumatic brain death induction 6 rats were lost, developing severe lung edema and subsequent failure of ventilation compared to none in the gradual model. Remarkable was the difference in mean arterial pressure before onset of brain death, followed by a considerably higher need of inotropic support in the explosive brain death model. In both groups patho-histological changes were found, but in the explosive model parenchyma injury was already pronounced immediately after confirmation of brain death as a result of a more pronounced edema formation in the explosive model. The over time increasing pro-inflammatory changes in gene expression were not substantially different between the models.

The results of this study suggest that donor lungs suffer more morphological injury after explosive onset of brain death, possibly making lungs unsuitable for transplantation, compared to gradual onset. However, findings in gene expression led us to conclude that if lungs are considered suitable for transplantation the outcome after transplantation should be independent of the etiology of brain death.

Introduction

Lung transplantations are commonly performed using lungs derived from brain dead organ donors, who died of an extensive central nervous system injury secondary to an event of trauma, hemorrhage or infarction [1]. At onset of brain death series of detrimental processes is initiated as a result of increased intracranial pressure. Initially, a massive increase of catecholamines in the blood occurs, also known as autonomic storm, this is accompanied by a significant increase in systemic vascular resistance (SVR) [2-4]. The sudden increase in systemic vascular resistance results in the pooling of a large proportion of the total blood volume in the cardio pulmonary vasculature. Shortly after the autonomic storm the SVR decreases again and the aortic blood flow either normalizes or in most of the subjects even results in hypotension [5].

Along with the hemodynamic changes brain death initiates an immune response in the peripheral organs [6]. Brain death related changes have been identified as underlying reason for inferior outcome after lung transplantation [7]. The lung seems to be more susceptible to brain death induced injury than other organ systems because from reported brain dead donors 90% of the kidneys and 70% of the livers were procured for transplantation in contrast to only 20% of the lungs in 2007 [8]. Lungs are selected dependent on their quality [9]. Reduced lung quality is the result of both, the early hemodynamic changes and the pro-inflammatory immune response which are also linked to the formation of pulmonary edema and increased pulmonary capillary leakage [10-12]. Nevertheless, more pronounced hemodynamic and hormonal changes have been described for the explosive brain death model [13, 14]. As a consequence of the more pronounced alterations in the explosive model ischemic injury in the donor hearts is more severe, explaining the inferior outcome after transplantation [14]. The hemodynamic changes in the explosive model have been suggested to impact the graft quality before as well as after transplantation [10, 15]. In difference to other organ systems clinical data are not conclusive whether cause of brain death affects lung transplantation outcome [16-19]. We investigated in an animal experiment if the etiology of brain death affects the quality of the donor lungs and whether this is due to differences in hemodynamics, immune response or both.

Materials and Methods

Animals

All rats were kept under clean conventional conditions, with a 12/12h light-dark cycle at 22 °C and were fed standard rat chow *ad libitum*. All animals received humane care in compliance with the Principles of Laboratory Animal Care (NIH Publication No.86-23, revised 1985) and the Dutch Law on Experimental Animal Care. The experiments were approved by the local animal care committee.

Experimental groups

Fisher (F344) rats weighing 270-300 g at the time of experiment were obtained from Harlan, Netherlands. In total seventy-two (excluding 6 animals lost after BD induction) rats were randomly assigned into three groups: 1) no intervention (control), 2) explosive (1 minute) or 3) gradual (30 minutes) brain death induction model. Animals in group 2 and 3 were sacrificed at four different time points: 30 minutes, 1 hour, 2 hours and 4 hours after onset of brain death.

Brain death model

Anaesthesia was induced with oxygen (1 l/min) /isoflurane 5% and reduced to oxygen/isoflurane 2% for continuation. Subcutaneously 2 ml of saline were administered before the start of surgery. Animals were tracheotomized in supine position, intubated with a 14G polyethylene tube and mechanically volume controlled ventilated with a Harvard apparatus (model 683). The ventilation was set at a tidal volume of 6.5 ml/kg of body weight (BW), PEEP of 3 cmH₂O and fraction of inspired oxygen (FiO₂) of 1 until the end of brain death induction when it was reduced to 0.5. The respiratory frequency was initially set at 120/min. and was throughout the experiment adjusted as needed to keep the ET-CO₂ between 20-22 mmHg.

The left femoral artery was cannulated and used for continuous monitoring of mean arterial pressure (MAP), while the left femoral vein was cannulated and used to stabilize the MAP above 80 mmHg by fluid boli (with a maximum of 1 ml per hour) of colloidal solution (HAES- steril 10%, Fresenius Kabi, Bad Homburg, Germany) and infusions of norepinephrin (0.01 mg/ml) as needed with a perfusor.

In all experimental groups, a fronto-lateral burr hole was drilled with a microdrill at 10,000 rpm with the boring head 9905 (Dremel, Breda, Netherlands) and a 4F fogarty catheter (Edwards Lifesciences LLC, Irvine, U.S.A.) inserted. Brain death was induced with 0.41 ± 0.5 ml distilled water by infusion of 4F fogarty catheter and expanded with a syringe perfusor pump (Terufusion Syringe Pump, model STC-

521). For the explosive model the fogarty catheter was expanded in 60 sec., for the gradual model in 30 min. To reduce muscle movements during BD induction 0.6 mg/kg of bodyweight rocuronium was given.

Body temperature was monitored rectally and kept at 38 °C with a heating pad. Brain death was confirmed by the absence of corneal reflexes 30 minutes after brain death induction. At the end of the observation period 0.1 mg/kg of BW succinylcholine was given i.v. just prior to organ harvest to prevent movement due to spinal reflexes. After this a laparo- and thoracotomy was performed and the aorta was punctured to withdraw blood for plasma analysis, the circulatory system was subsequently flushed with 40 ml of cold saline after incision of the inferior cava vein and the lung was harvested, after inflating the lung with 2 ml of air with a syringe and clamping of the trachea. Control group lungs were not inflated since the animals were not intubated. Lung-parts were fixed with 4% of formalin for histological analysis or snap frozen for wet-dry ratio and molecular biology analysis.

Wet-Dry ratio

The weights of the primarily in Eppendorf tubes snap frozen right middle lung lobes were measured before and after they were placed for 24 hrs at 100 °C on aluminum foil. The W/D ratio was calculated ((weight before drying at 100 °C – alu foil) / (weight after drying at 100 °C – alu foil) = Wet-Dry ratio) and is given as the mean ± standard deviation.

RNA isolation and reverse transcriptase Polymerase-Chain Reaction

Total RNA was isolated from snap frozen lung tissue using Trizol (Invitrogen Life Technologies, Breda, Netherlands) according to the manufacturer's instructions. Guanidine thiocyanate contaminated samples, identified by E260/E230 ratio below 1.6 with the nanodrop 1000 spectrophotometer, were purified before continuation. The integrity of total RNA was analyzed by gelelectrophoresis. To remove genomic DNA total RNA was treated with DNase I (Invitrogen, Breda, Netherlands). Of this 1 µg RNA was transcribed into cDNA using M-MLV (Moloney murine leukemia virus) Reverse Transcriptase (Invitrogen, Breda, Netherlands) in the presence of dNTPs (Invitrogen, Breda, Netherlands), after an initial incubation with Oligo-dT primers (Invitrogen, Breda, Netherlands).

Gene expression analyses were performed at mRNA level by TaqMan low density array (TLDA). Designed primer sets (Table 1) were loaded with 5 µl cDNA (2ng/µl) and SYBR green (Applied Biosystems) into a well of a TaqMan low density Array (TLDA) card. Amplification and detection were performed with the ABI Prism 7900-HT Sequence Detection System (Applied Biosystems) measuring the emission of

SYBR green. Each sample was measured in triplicate and a pool of sample cDNA served as calibrator. PCR reaction consisted of 40 cycles at 95 °C for 15 sec. and 60 °C for 60 sec. after initiation for 2 min. at 50 °C and 10 min. at 95 °C. A dissociation curve analysis was performed for each reaction to ensure the amplification of specific products.

Gene expression was normalized with the mean of the housekeeping genes PPIA and ELf2b1, and gene expression values calculated with the $\Delta\Delta C_t$ method [20].

Table 1. Designed PCR primers

Primer	Gene	Primer	Amplicon (bp)
Tnf	Tumor necrosis factor	5'-AGGCTGTCGCTACATCACTGAA-3' 5'-TGACCCGTAGGGCGATTACA-3'	67
Il6	Interleukin 6	5'-CCAACCTCCAATGCTCTCCTAATG-3' 5'-TTCAAGTGCTTTCAAGAGTTGGAT-3'	89
Cinc1	Chemokine (C-x-C motif) ligand 1	5'-TGGTTCAGAAGATTGTCCAAAAGA-3' 5'-ACGCCATCGGTGCAATCTA-3'	78
Ccl1 (Mcp1)	Chemokine (C-C motif) ligand 2	5'-CTTTGAATGTGAACCTTGACCCATAA-3' 5'-ACAGAAGTGCTTGAGGTGGTTGT-3'	78
Vcam1	Vascular adhesion molecule 1	5'-TGTGGAAGTGTGCCCCGAAA-3' 5'-ACGAGCCATTAACAGACTTTAGCA-3'	84
Gapdh	Glyceraldehyde-3-phosphate dehydrogenase	5'-GTATGACTCTACCCACGGCAAGTT-3' 5'-GATGGGTTTCCCGTTGATGA-3'	79
Ppia	Peptidylprolyl isomerase A	5'-TCTCCGACTGTGGACAACTCTAATT-3' 5'-CTGAGCTACAGAAGGAATGGTTTGA-3'	76
Eif2b1	Eukaryotic translation initiation factor 2B	5'-ACCTGTATGCCAAGGGCTCATT-3' 5'-TGGGACCAGGCTTCAGATGT-3'	77

Histological scoring

Lungs were fixed in 4% formalin, embedded in paraffin and cut in four- μ m-thick slices, subsequently stained with haematoxylin-eosin. A five-point semiquantitative severity-based scoring system was used as previously described [21]. The pathological findings were graded as negative = 0, slight = 1, moderate = 2, high = 3, and severe = 4. The amount of intra- and extra-alveolar haemorrhage, intra-alveolar edema, inflammatory infiltration of the interalveolar septa and airspace and overinflation were rated. Deviating from the original scoring scheme erythrocyte accumulation below the pleura was scored as 1 = present or 0 = not present. The scoring variables were added and a histological total lung injury score per slide was calculated after morphological examination was performed simultaneously by two blinded investigators. For this, a conventional light microscope was used at a magnification of 200 across 10 random, non-coincidental microscopic fields.

Plasma analysis

The collected blood was immediately placed into EDTA-tubes after harvest and centrifuged at 10,000 rpm at 4 °C. Supernatant was transferred into sterile Eppendorf tubes and stored until further use at – 80 °C. Analysis for troponin and creatinine kinase (CK-MB) were performed in the clinical laboratory.

Statistical Analysis

A two way ANOVA was conducted to examine the effects of brain death model and time on physiological parameters, wet-dry-ratio, RTD- PCR, histological scoring, total volume administration, NA, CK-MB and Troponin. Outliers were assessed by inspection of the boxplot. Normality of the data distributions were visually inspected using probability-probability plot (P-P plot). Not normally distributed data were transformed by the natural logarithm. To correct zero values in total volume administration and histological scoring 0.1 was added before performing the natural logarithm. Mean arterial pressure during brain death induction, Mcp1 and Vcam1 showed in the boxplot a quadratic relationship with time, for that reason data were transformed by centering the time before end of brain death induction for MAP at -10 and Vcam1 and Mcp1 at 1.5 h, subsequently the time was squared before proceeding. For not normally distributed data (Paw, HR, total volume, NA, wet-dry-ratio, edema and pleura infarction) the Mann-Whitney test was used to determine differences between brain death models. The MAP was not normally distributed at end of brain death induction and after 4hrs of brain death therefore a Mann-Whitney test was used comparing the two brain death models. Data from animals with failure of hemodynamic stabilization were excluded from analysis. Presented data are given as mean \pm standard deviation (SD) if not mentioned otherwise. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using IBM SPSS 22.

Results

Failure of hemodynamic stabilization

Fisher rats (F344) were randomly assigned to control or the brain death models with subgroups of different stabilization periods. In the explosive brain death arm 6 animals were lost immediately after the induction of brain death since ventilation was not feasible (excluded from analysis and replaced), while no animal was lost in the gradual brain death model. At dissection of the thoracic cavity in 5 out of 6 animals a fulminant lung edema became visible.

Physiological data

Of the three groups, only the two brain death groups were ventilated and stabilized during which time physiological data were collected. The parameters pulmonary inspiratory pressure, heart rate and mean arterial pressure of each brain death model were taken together as available for the given time points and analyzed (Table 2). There was no difference in pulmonary inspiratory pressure between the models but the heart rate was elevated in the explosive brain death model.

Table 2. Physiological parameters selected in the brain dead donor groups.

	BL		BD 0.5h		BD 1h		BD 2h		BD 4h	
	EM n = 30	GM n = 29	EM n = 27	GM n = 19	EM n = 22	GM n = 21	EM n = 15	GM n = 12	EM n = 5	GM n = 6
Paw (cmH₂O)	8 ± 1	8 ± 1	9 ± 1	9 ± 1	9 ± 1	9 ± 1	10 ± 2	9 ± 2	11 ± 2	10 ± 2
HR (/min.)	345 ± 33	352 ± 48	426 ± 48	408 ± 56	425 ± 52	375 ± 58	415 ± 48	375 ± 58	387 ± 44	385 ± 34

The given values are presented as mean ± standard error of the mean.

BD 0.5h– 4h– brain dead group with period of ventilation and circulatory stabilization time; BL– before brain death induction; EM– explosive brain death induction model, expansion of the fogarty catheter in 60 sec. with a pump; GM– gradual brain death induction model, expansion of the fogarty catheter over a period of 30 min. with a pump; HR– heart rate (beats/min.); MAP– mean arterial pressure; Paw– pulmonary airway pressure.

The mean arterial pressure in the explosive model was significantly higher during twenty minutes before end of brain death induction compared to the gradual model. During the initial phase of hemodynamic stabilization was the MAP higher in the explosive model, while there was no difference between groups after four hours of stabilization (Figure 1). In contrast to this, the need of total volume administration in the explosive model was higher compared to the gradual model (1.2 ± 1.1 ml (EM) vs. 0.6 ± 0.5 ml (GM); $p < 0.05$). Nevertheless, there were no relevant differences in Wet-Dry ratio between the groups including the control group.

The time of brain death induction does not influence the investigated proinflammatory gene expression during 4 hrs. of brain death

In both brain death models the pro-inflammatory cytokine gene expression of Tnf and Il6 was induced, but levels did not reach significance between the two models. Gene expression of the Il8 like Cinc1 was unaffected by groups and time. For Mcp1 and Vcam1 an increase in a quadratic regression was noted over time with the time centered at 1.5 hrs. For Gapdh a trend towards significance ($p < 0.053$) was found between groups. For that reason, different housekeeping genes were chosen for further analysis (Table2).

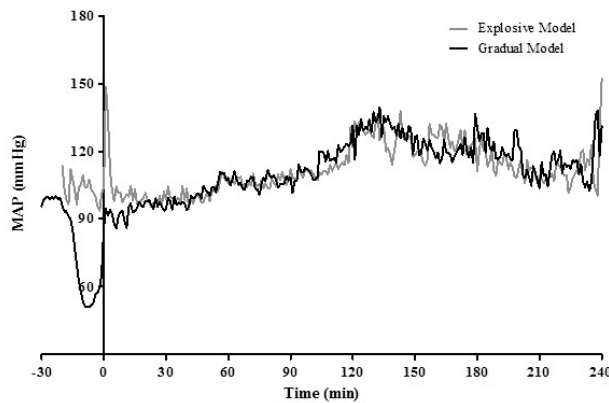


Figure 1. Mean arterial pressure overtime. Expansion of the fogarty catheter and induction of brain death was finished at time point zero in both models. After end of brain death induction all animals were stabilized above a mean arterial pressure of 80 mmHg. The presented mean arterial pressure data are depicted as mean.

Table 3. Changes in pro-inflammatory cytokine gene expression after different durations of brain death.

RTD-PCR	BD 0.5h			BD 1h		BD 2h		BD 4h	
	Control n = 8	EM n = 8	GM n = 8	EM n = 8	GM n = 8	EM n = 8	GM n = 8	EM n = 8	GM n = 8
Tnf	0.02 ± 0.00	1.05 ± 0.36	0.60 ± 0.32	0.73 ± 0.19	0.91 ± 0.32	0.75 ± 0.22	0.88 ± 0.34	1.39 ± 0.65	1.52 ± 0.80
Il6	0.01 ± 0.02	0.72 ± 1.49	0.57 ± 0.60	0.75 ± 0.40	1.17 ± 0.93	3.62 ± 2.78	2.53 ± 2.99	7.22 ± 6.74	12.20 ± 9.49
Cinc1	0.03 ± 0.03	0.69 ± 0.97	0.41 ± 0.33	0.92 ± 0.60	1.67 ± 0.92	1.58 ± 1.36	1.42 ± 1.11	1.08 ± 1.01	1.38 ± 0.76
Mcp1	0.02 ± 0.02	0.35 ± 0.71	0.10 ± 0.06	0.2 ± 0.10	0.5 ± 0.21	1.22 ± 0.83	1.24 ± 0.26	1.62 ± 0.60	2.52 ± 1.23
Vcam1	0.14 ± 0.04	0.55 ± 0.25	0.98 ± 0.39	1.33 ± 0.36	1.59 ± 0.26	0.99 ± 0.38	1.27 ± 0.27	0.81 ± 0.46	1.08 ± 0.34
GAPDH	0.82 ± 0.48	0.98 ± 0.39	1.26 ± 0.39	1.44 ± 0.28	1.52 ± 0.24	1.33 ± 0.52	1.75 ± 0.58	1.83 ± 0.23	1.82 ± 0.74

The presented values are mean ± standard deviation (SD).

BD 0.5h– 4h– brain dead group with period of ventilation and circulatory stabilization time; Control– immediately sacrificed without intervention; Ccl2– chemokine (C-C motif) ligand 2, also known as Mcp1 (Monocyte chemoattractant protein1); Cxcl1– chemokine (C-x-C motif) ligand 1, also known as Cinc1 (Cytokine induced neutrophil chemoattractant1); EM– explosive brain death induction model, expansion of the fogarty catheter in 60 sec. with a pump; GM– gradual brain death induction model, expansion of the fogarty catheter over a period of 30 min. with a pump; Il6– interleukin 6; RTD-PCR– real-time detection PCR; Tnf– tumor necrosis factor; Vcam1– vascular adhesion molecule 1

Explosive brain death induction results in more severe lung injury then gradual brain death induction on a histological level

Total lung injury score was higher in the explosive brain death model than in the gradual brain death model, as well as changes were significant over time. This was the result of a more pronounced hemorrhagic infarcted lung tissue, edema and pleural infarction in the explosive model compared to the gradual brain death model (Figure 2 + Table 4).

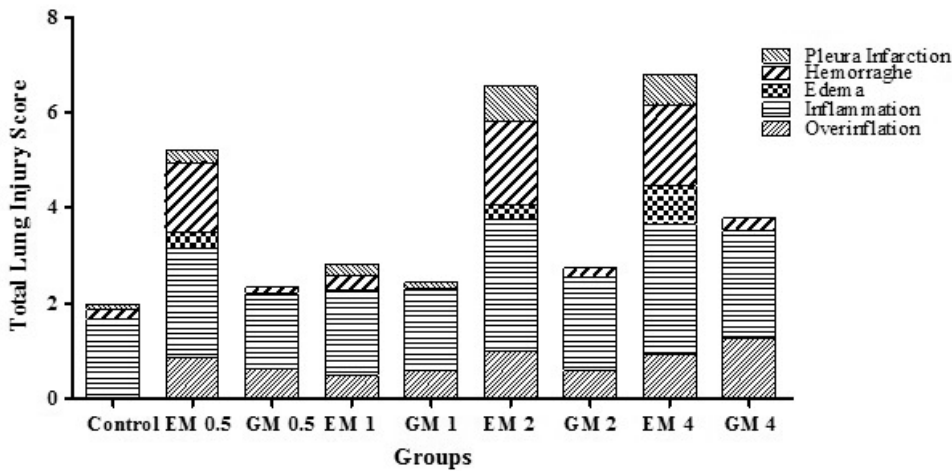


Figure 2. Histological total lung injury score. Formalin fixed, paraffin embedded and haematoxylin-eosin stained left lung lobe slices were scored for the extent of intra- and extra-alveolar haemorrhage, intra-alveolar edema, inflammatory infiltration of the interalveolar septa and airspace and overinflation. The pathological findings were graded as negative = 0, slight = 1, moderate = 2, high = 3, and severe = 4. Deviating from the published original scoring scheme erythrocyte accumulation below the pleura was scored as 1 = present or 0 = not present. The sum of variables gave the total lung injury score. Morphological examination was performed in a blinded fashion by 2 investigators, using a conventional light microscope at a magnification of 200 across 10 random, non-coincidental microscopic fields. All values are presented as mean.

Control- no intervention; EM- explosive brain death induction within 1 minute by expansion of a intracranial fogarty catheter; GM- gradual brain death induction over a period of 30 minutes; 0.5- 30 minutes of donor stabilization; 1/2/4- hours of donor management.

Need of inotropic support and release of heart injury markers are more pronounced in the explosive model

To ensure sufficient oxygenation of the solid organs, brain dead donors are stabilized at a mean arterial pressure above 80 mmHg, for this inotropic substances are being used in the clinic. We chose to give 0.01 mg/ ml noradrenalin (NA) as needed with a perfusor. There was a distinct difference between the two models. The need of NA was higher in the explosive model compared to the gradual model ($p < 0.005$).

Both, brain death and inotropic support may have substantial effect on the heart and subsequently on the lung. For that reason, we analyzed two sensitive markers for heart injury- CK-MB and troponin. The release of CK-MB into the plasma was higher in the explosive model compared to the gradual model, however, did not change over time. Correlating to the CK-MB levels did the troponin levels differ between the groups. However, there was also a respective increase over time (Table 5).

Table 4. Histological lung injury score.

Histological Score	BD 0.5h			BD 1h			BD 2h			BD 4h		
	Control n = 8	EM n = 8	GM n = 8	EM n = 8	GM n = 8	EM n = 8	EM n = 8	GM n = 8	EM n = 8	GM n = 8	EM n = 8	GM n = 8
Hemorrhage	0.2 ± 0.1	1.5 ± 1.8	0.1 ± 0.2	0.3 ± 0.6	0.0 ± 0.0	1.7 ± 1.5	0.0 ± 0.0	0.2 ± 0.2	1.7 ± 0.5	0.3 ± 0.3	0.3 ± 0.3	0.3 ± 0.3
Edema	0.0 ± 0.0	0.3 ± 0.3	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.3 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 1.2	0.0 ± 0.0	0.8 ± 1.2	0.0 ± 0.0
Inflammation	1.6 ± 0.6	2.3 ± 0.7	1.6 ± 0.9	1.7 ± 0.5	1.7 ± 0.4	2.8 ± 0.6	1.7 ± 0.4	2.0 ± 0.7	2.7 ± 0.9	2.2 ± 1.1	2.7 ± 0.9	2.2 ± 1.1
Over-inflation	0.0 ± 0.1	0.9 ± 0.6	0.6 ± 0.4	0.5 ± 0.3	0.6 ± 0.4	1.0 ± 0.5	0.6 ± 0.4	0.6 ± 0.3	0.9 ± 0.7	1.3 ± 0.7	0.9 ± 0.7	1.3 ± 0.7
Pleura	0.1 ± 0.4	0.3 ± 0.5	0.0 ± 0.0	0.3 ± 0.5	0.1 ± 0.4	0.8 ± 0.5	0.1 ± 0.4	0.0 ± 0.0	0.6 ± 0.5	0.0 ± 0.0	0.6 ± 0.5	0.0 ± 0.0
Infarction												
Total	2.0 ± 0.6	5.2 ± 3.0	2.3 ± 1.2	2.8 ± 1.1	2.4 ± 0.7	6.5 ± 2.4	2.4 ± 0.7	2.7 ± 0.6	6.8 ± 3.3	3.8 ± 1.5	6.8 ± 3.3	3.8 ± 1.5

The values presented are mean ± standard deviation (SD).

Table 5. Administered total inotropic support during donor management and heart muscle injury markers troponin and CK-MB.

	BD 0.5h			BD 1h			BD 2h			BD 4h		
	Control n = 8	EM n = 8	GM n = 8	EM n = 8	GM n = 8	EM n = 8	EM n = 8	GM n = 8	EM n = 8	GM n = 8	EM n = 8	GM n = 8
NA	-	0.8 ± 1.0	0.0 ± 0.0	0.6 ± 0.8	0.1 ± 0.1	1.1 ± 1.6	0.1 ± 0.1	0.1 ± 0.2	0.3 ± 0.58	0.2 ± 0.4	0.3 ± 0.58	0.2 ± 0.4
CK-MB	409 ± 175	1656 ± 1046	678 ± 152	864 ± 300	731 ± 225	1091 ± 670	830 ± 258	830 ± 258	703 ± 66	671 ± 227	703 ± 66	671 ± 227
Troponin	22 ± 39	31 ± 11	28 ± 17	74 ± 45	34 ± 16	135 ± 90	59 ± 33	59 ± 33	131 ± 126	130 ± 152	131 ± 126	130 ± 152

The presented values are the mean ± standard deviation (SD).

BD 0.5h–4h– brain dead group with period of ventilation and circulatory stabilization time; CK-MB– Creatine kinase (myocardium); Control– immediately sacrificed without intervention; EM– explosive brain death induction model, expansion of the fogarty catheter in 60 sec. with a pump; GM– gradual brain death induction model, expansion of the fogarty catheter over a period of 30 min. with a pump; NA– Noradrenalin

Discussion

This is to our knowledge, the first investigation comparing the effect of explosive and gradual brain death induction models on lung graft quality over different time periods in rats. Both models resulted in the decrease of graft quality over time, however, more pronounced in the explosive brain death induction model during the initial phase. This difference was observed in higher mortality, increase of pulmonary inspiratory pressure, more need of hemodynamic support and pronounced patho-histological changes. In contrast to this, no differences were found in the investigated proinflammatory gene expression.

Since in kidney and heart transplantation the cause of brain death seems to have a substantial influence on the early outcome after transplantation [18, 19], a number of studies have investigated the effect of cause of brain death in lung transplantation. While they failed to show a correlation between cause of brain death and outcome [16, 22, 23], it was noted that donors with traumatic brain injury had a higher incidence in severity of rejection episodes though this had no effect on the survival [17]. However, these studies did not analyse whether the cause of brain death affects the availability of donor organs.

In a comparative study, with emphasis on the heart, it has been shown that the two brain death induction models differ in the development of mean arterial pressure during brain death induction. The acute increase in mean arterial pressure in the explosive brain death induction model is compared to a more tempered development in the gradual model, and is considered to be the result of a more pronounced sympathetic discharge, followed by a more profound hypotension [14]. Sudden increase in mean arterial pressure causes the rupture of the capillary-alveolar membrane and disruption of the barrier integrity. Preventing the hypertensive response to explosive brain death induction limited the inflammatory immune response and prevented changes in capillary-alveolar membrane integrity [10]. This could explain, why acute cerebral insult and brain death have been associated with the onset of pulmonary edema [5, 24, 25], while it is rare in subarachnoid hemorrhage [26], which causes a gradual increase of intracranial pressure. Additionally, the loss of capillary integrity is accompanied by hemorrhage [5]. The hemodynamic pattern during the initial phase between our two brain death induction models respectively differs and may explain the difference we found in loss of animals during the initial phase after brain death induction. After the induction phase the mean arterial pressure was stabilized in our set up by utilization of HAES and noradrenalin. Administration of exogenous noradrenalin has been associated with graft deterioration in other solid organ systems [27], however, in the lung the prevention of hypotensive collapse using

noradrenalin correlated with reduced edema and inflammation [28]. In this setting, intrinsic catecholamine levels up on brain death induction were not determined nor histological myocardial changes. However, it is expected that the increase in troponin and CK-MB are the results of more pronounced intrinsic catecholamine release [14].

While there seems to be a correlation between hemodynamic changes and the inflammatory immune response during brain death [10, 15, 28], it is not the only reason for the inflammatory immune response found in brain dead donor lungs. Exposing living controls to blood withdrawn from brain dead donors in the absence of hemodynamic changes causes an inflammatory response [6]. The origin of this systemic inflammation has not been identified yet [6, 29].

In conclusion, the results of this study suggest that donor lungs suffer more morphological injury after explosive onset of brain death. As a consequence, less lungs from donors with acute onset of brain death might be considered suitable for lung transplantation. Nonetheless, this has not been investigated in this study, nor to our knowledge clinically. However, findings in gene expression lead us to conclude that the outcome after transplantation should be independent of the etiology of brain death.

References

1. Pratschke, J., et al., *Accelerated rejection of renal allografts from brain-dead donors*. *Ann Surg*, 2000. **232**(2): p. 263-71.
2. Cooper, D.K., D. Novitzky, and W.N. Wicomb, *Hormonal therapy in the brain-dead experimental animal*. *Transplant Proc*, 1988. **20**(5 Suppl 7): p. 51-4.
3. Cooper, D.K., D. Novitzky, and W.N. Wicomb, *The pathophysiological effects of brain death on potential donor organs, with particular reference to the heart*. *Ann R Coll Surg Engl*, 1989. **71**(4): p. 261-6.
4. Powner, D.J., et al., *Changes in serum catecholamine levels in patients who are brain dead*. *J Heart Lung Transplant*, 1992. **11**(6): p. 1046-53.
5. Novitzky, D., et al., *Pathophysiology of pulmonary edema following experimental brain death in the chacma baboon*. *Ann Thorac Surg*, 1987. **43**(3): p. 288-94.
6. Takada, M., et al., *Effects of explosive brain death on cytokine activation of peripheral organs in the rat*. *Transplantation*, 1998. **65**(12): p. 1533-42.
7. Zweers, N., et al., *Donor brain death aggravates chronic rejection after lung transplantation in rats*. *Transplantation*, 2004. **78**(9): p. 1251-8.
8. Van Raemdonck, D., et al., *Lung donor selection and management*. *Proc Am Thorac Soc*, 2009. **6**(1): p. 28-38.
9. Orens, J.B., et al., *A review of lung transplant donor acceptability criteria*. *J Heart Lung Transplant*, 2003. **22**(11): p. 1183-200.
10. Avlonitis, V.S., et al., *The hemodynamic mechanisms of lung injury and systemic inflammatory response following brain death in the transplant donor*. *Am J Transplant*, 2005. **5**(4 Pt 1): p. 684-93.
11. Edmonds, H.L., Jr., et al., *Effects of aerosolized methylprednisolone on experimental neurogenic pulmonary injury*. *Neurosurgery*, 1986. **19**(1): p. 36-40.
12. Theodore, J. and E.D. Robin, *Speculations on neurogenic pulmonary edema (NPE)*. *Am Rev Respir Dis*, 1976. **113**(4): p. 405-11.
13. Kolkert, J.L., et al., *The gradual onset brain death model: a relevant model to study organ donation and its consequences on the outcome after transplantation*. *Lab Anim*, 2007. **41**(3): p. 363-71.
14. Shivalkar, B., et al., *Variable effects of explosive or gradual increase of intracranial pressure on myocardial structure and function*. *Circulation*, 1993. **87**(1): p. 230-9.
15. Avlonitis, V.S., et al., *Early hemodynamic injury during donor brain death determines the severity of primary graft dysfunction after lung transplantation*. *Am J Transplant*, 2007. **7**(1): p. 83-90.
16. Wauters, S., et al., *Donor cause of brain death and related time intervals: does it affect outcome after lung transplantation?* *Eur J Cardiothorac Surg*, 2011. **39**(4): p. e68-76.
17. Ciccone, A.M., et al., *Does donor cause of death affect the outcome of lung transplantation?* *J Thorac Cardiovasc Surg*, 2002. **123**(3): p. 429-34; discussion 434-6.
18. Pessione, F., et al., *Multivariate analysis of donor risk factors for graft survival in kidney transplantation*. *Transplantation*, 2003. **75**(3): p. 361-7.
19. Cohen, O., et al., *Donor brain death mechanisms and outcomes after heart transplantation*. *Transplantation proceedings*, 2007. **39**(10): p. 2964-9.
20. Schmittgen, T.D. and K.J. Livak, *Analyzing real-time PCR data by the comparative C(T) method*. *Nat Protoc*, 2008. **3**(6): p. 1101-8.
21. Krebs, J., et al., *Open lung approach associated with high-frequency oscillatory or low tidal volume mechanical ventilation improves respiratory function and minimizes lung injury in healthy and injured rats*. *Crit Care*, 2010. **14**(5): p. R183.
22. Waller, D.A., et al., *Does the mode of donor death influence the early outcome of lung transplantation? A review of lung transplantation from donors involved in major trauma*. *J Heart Lung Transplant*, 1995. **14**(2): p. 318-21.
23. Ganesh, J.S., et al., *Donor cause of death and mid-term survival in lung transplantation*. *J Heart Lung Transplant*, 2005. **24**(10): p. 1544-9.
24. Simmons, R.L., et al., *Respiratory insufficiency in combat casualties. II. Pulmonary edema following head injury*. *Ann Surg*, 1969. **170**(1): p. 39-44.

- R1 25. Rogers, F.B., et al., *Neurogenic pulmonary edema in fatal and nonfatal head injuries*. J Trauma, 1995.
R2 39(5): p. 860-6; discussion 866-8.
- R3 26. Friedman, J.A., et al., *Pulmonary complications of aneurysmal subarachnoid hemorrhage*. Neurosurgery,
R4 2003. 52(5): p. 1025-31; discussion 1031-2.
- R5 27. Ueno, T., C. Zhi-Li, and T. Itoh, *Unique circulatory responses to exogenous catecholamines after brain*
R6 *death*. Transplantation, 2000. 70(3): p. 436-40.
- R7 28. Rostron, A.J., et al., *Hemodynamic resuscitation with arginine vasopressin reduces lung injury after*
R8 *brain death in the transplant donor*. Transplantation, 2008. 85(4): p. 597-606.
- R9 29. Skrabal, C.A., et al., *Organ-specific regulation of pro-inflammatory molecules in heart, lung, and kidney*
R10 *following brain death*. J Surg Res, 2005. 123(1): p. 118-25.
- R11
- R12
- R13
- R14
- R15
- R16
- R17
- R18
- R19
- R20
- R21
- R22
- R23
- R24
- R25
- R26
- R27
- R28
- R29
- R30
- R31
- R32
- R33
- R34
- R35
- R36
- R37
- R38
- R39